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Use of Operant Performance to Guide and Evaluate Medical Treatment in an Adult Male Cynomolgus Macaque (*Macaca fascicularis*)

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A 6-y-old male cynomolgus macaque presented with noticeable swelling of the left forearm and signs of discomfort, as indicated by nonuse of the arm even in a behavioral task that he previously had been well-motivated to perform. Examination under anesthesia revealed lacerations to the arm. Radiography of the forearm showed no fractures, indicating that the damage was limited to soft tissue. The daily operant behavioral session assessed the amount of force the monkey emitted when touching the screen with the affected arm and how long each touch was sustained. We then used these parameters (force and duration of touch) as objective measures of putative pain relief and recovery of function to guide the medical treatment. The affected monkey received ketoprofen, buprenorphine, or their combination but continued to perform poorly during daily operant behavioral sessions. Only after treatment with dexamethasone did performance return to preinjury levels, suggesting inflammation near the radial or ulnar nerve. These findings indicate that performance of a trained operant task performance can be useful in guiding medical treatment, evaluating pain relief, and objectively monitoring health in laboratory animals.

Abbreviation: TRD, temporal response differentiation.

Operant behavior is behavior that is shaped and maintained by its consequences in a given context and encompasses behavioral repertoires as diverse as verbal behavior (for example, reading, writing, speaking) and refined motor performances (for example, driving a car, brushing one's teeth, playing a musical instrument). The ubiquity of operant behavior and its importance for survival make the study of operant behavior important in its own right. For several decades, the use of operant behavior in laboratory settings for the evaluation of the effects of drugs (and other manipulations) has proven quite useful, and the field of behavioral pharmacology is well recognized.[2,3,5,6,10,11] Less well-recognized is the use of operant behavior to improve animal welfare in laboratory settings and to guide effective therapies and veterinary practices. The present study is a case report of medical treatment guided by the changes in operant performance in a highly trained male cynomolgus macaque (*Macaca fascicularis*).

Case Report

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*[4] and the Animal Welfare Act of 1966, as amended.[1]

Subject. The subject was a 6-y-old male cynomolgus macaque weighing 6.0 kg. The monkey was weighed weekly and fed enough food daily (no. 8714 Teklad 15% Monkey Diet, Harlan Laboratories, Indianapolis, IN, and fresh fruit and vegetables) to maintain body weight at approximately 95% of free-feeding levels. The monkey was housed individually in a stainless steel squeeze-back cage (61 cm × 71 cm × 86 cm), with water available ad libitum and constant visual and auditory contact with other cynomolgus monkeys. The colony was maintained under a 12:12-h light:dark cycle (lights on, 0600) and at 20 to 22 °C, with a relative humidity of 50% ± 15%. The behavioral sessions generally occurred 5 d each week (Monday through Friday), and this monkey had received extensive training over 2 y on the task prior to his injury.

Behavioral apparatus. The subject was tested unrestrained in its home cage by using an aluminum intelligence panel affixed to the front of the cage. Unhindered access to the panel was achieved by securing the cage door in the open position with a cord. The panel consisted of a touch screen monitor (38-cm flat-panel LCD, model 1537L, SecureTouch Surface Acoustic Wave, ELO TouchSystems, Menlo Park, CA) and a food cup equipped with a white light that signaled food pellet delivery. A clear acrylic door attached to a microswitch (requiring 1.7 N force to operate) measured entries into the food cup. The pellet dispenser delivered 190-mg banana-flavored pellets (F0035, Bio-Serv, Frenchtown, NJ) into the food cup, and a notebook computer (Latitude D620, Dell, Round Rock, TX; running Windows XP, Microsoft, Redmond, WA) controlled experimental events and collected data by using a custom Visual Basic 6.0 program. A screen touch of greater than approximately 0.69 N force was counted as a response. In addition, this touch screen provided data on the force of each response, measured every 40 ms and ranging from 0 to 255 in arbitrary force units. Pellet dispenser and LED operation and detection of hopper door switch closures were managed by using a USB relay I/O interface (ADU208, Ontrak Control Systems, Sudbury, Ontario, Canada) connected to the computer.

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Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
9/27 Ketoprofen	9/28 Ketoprofen	9/29 Ketoprofen	9/30 Ketoprofen	10/1 Ketoprofen	10/2 Ketoprofen	10/3 No medication
10/4 No medication	10/5 Ketoprofen	10/6 Ketoprofen	10/7 Ketoprofen	10/8 Buprenorphine	10/9 Ketoprofen Buprenorphine	10/10 Ketoprofen Buprenorphine
10/11 Ketoprofen Buprenorphine	10/12 Ketoprofen Buprenorphine	10/13 Ketoprofen Buprenorphine	10/14 No medication	10/15 Dexamethasone (1 mg/kg)	10/16 Dexamethasone (0.75 mg/kg)	10/17 Dexamethasone (0.5 mg/kg)
10/18 Dexamethasone	10/19 Dexamethasone	10/20 Dexamethasone	10/21 Dexamethasone	10/22 Dexamethasone	10/23 Dexamethasone	10/24 Dexamethasone
10/25 Dexamethasone	10/26 Dexamethasone	10/27 Dexamethasone	10/28 Dexamethasone	10/29 No medication	10/30 Dexamethasone	10/31 No medication
11/1 Dexamethasone	11/2 All medication discontinued					

Figure 1. Calendar of pharmaceutical treatment of the injured monkey. Doses: ketoprofen, 2.0 mg/kg IM in the morning; buprenorphine, 0.01 mg/kg IM in the evening; dexamethasone, 0.25 mg/kg IM (except where noted otherwise).

Behavioral assessment. The temporal response differentiation (TRD) task requires the production of a response duration within a very narrow band (in this case, between 4.0 and 5.6 s) and has been shown to be particularly sensitive to the performance-altering effects of drugs.[7,8] The time between pressing and releasing the stimulus on the touch screen defines this response duration. Because the animal must press the touch screen within a small area and hold its finger(s) there for a prescribed time, the task simultaneously assesses manual dexterity, concentration, and perceived passage of time. Each session lasted approximately 2.5 h, during which a maximum of 180 trials were presented, with a 40-s interval separating each trial (during which the screen was blank and responses produced no programmed consequences). Response duration and response force on each trial served as the primary dependent measures.

Injury description. On Sunday, 27 September 2010, veterinary caretakers noted that this monkey's left arm was swollen and he was holding it against his body in a guarded position. Because the monkey had already eaten, physical examination was scheduled for the next day, and the monkey was given ketoprofen (2.0 mg/kg IM) to treat possible pain. On Monday, 28 September, behavioral performance was impaired greatly. Veterinary medical examination later that day showed lacerations on the dorsal (3-cm laceration) and the ventral (2-cm laceration) left forearm with swelling, good scabbing, no bleeding, and minimal redness. There was no palpable instability of the long bones of the arm, and X-rays confirmed the absence of fractures from humerus to fingertip. The diagnosis was laceration and secondary loss of limb function due to suspected self-inflicted injury. The wounds were clipped and washed, but due to the time since injury, the wound was left to heal on its own rather than by suturing the wound closed.

Treatment strategy and drug selection. Drugs were administered in the afternoon (Figure 1), except where noted, to minimize potential interference with operant performance. Initially, ketoprofen (2.0 mg/kg IM) was given for pain relief. However, the monkey's performance on the operant behavioral task did not improve. On 08 October, given that TRD performance had still not recovered to preinjury levels, evening administration of buprenorphine (0.01 mg/kg IM) was added to the treatment regimen of ketoprofen (2.0 mg/kg IM) administration in the morning. Although 2 wk is the theoretical healing window for soft tissue,[9] behavioral performance on the TRD task had still not improved by 15 October (more than 14 d after injury). Therefore, because of the veterinarian's concern regarding possible damage to the monkey's radial or ulnar nerve, previous treatments were discontinued, and glucocorticosteriod treatment was implemented. Specifically, a high loading dose of dexamethasone (1.0 mg/kg) was administered on 15 October (Figure 1) and then reduced gradually to 0.25 mg/kg on 18 October, with a final injection administered on 01 November. Tapering the dosage and prolonging the interval between doses were implemented to avoid potential problems with the adrenal glands after withdrawal from corticosteroid therapy. Behavioral performance on the TRD task returned to baseline (preinjury) levels soon after dexamethasone therapy was initiated and remained there even after discontinuation of the drug; therefore, no further treatments were warranted.

Data analysis. Duration and force were evaluated across the various phases of observation (baseline, injury, dexamethasone treatment, and after treatment) by using separate one-way repeated-measures ANOVA (SigmaStat for Windows version 2.03, SPSS, Chicago, IL) and Bonferroni posttests for all pairwise comparisons. A significance level of P less than 0.05 was used for all tests.

Results

Prior to the monkey's injury, baseline performance (Figure 2) revealed consistent mean response durations that approximated the lower limit of the reinforced band (that is, 4 s). One-way repeated-measures ANOVA revealed a significant effect of injury condition ($F[3,23] = 46.67$, $P < 0.001$). After the injury was discovered, mean response durations approximated 0.5 s or less, well below the reinforced band. Treatment with ketoprofen or the ketoprofen-buprenorphine combination failed to increase mean response duration (Figure 3). In contrast, treatment with dexamethasone increased mean response duration to about 2.25 s,

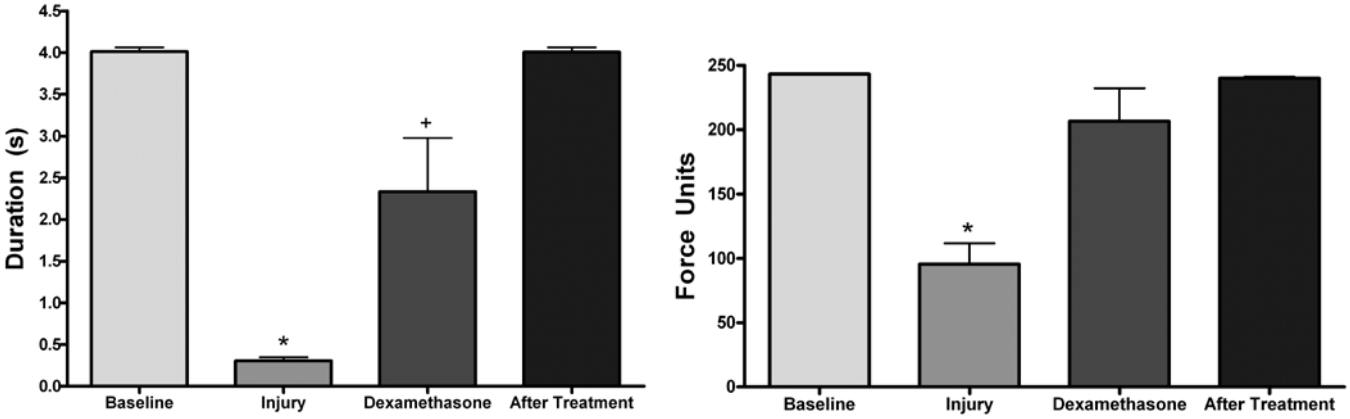

Figure 2. Mean response duration (s) for all attempts during TRD sessions (left panel) and mean force emitted for the first 10 trials of each TRD session (right panel). *, Value significantly ($P < 0.05$) different from those for baseline, dexamethasone, and after treatment; +, value significantly ($P < 0.05$) different from that for injury.

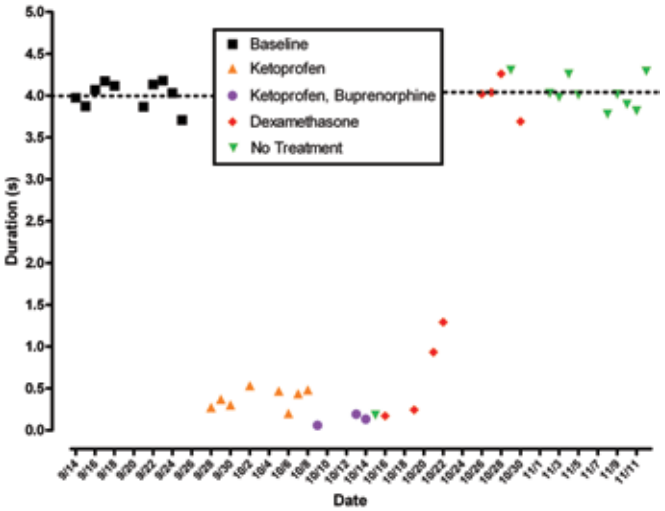

Figure 3. Mean response duration (s) per session throughout the course of the injury and treatment.

still shorter ($P < 0.05$) than baseline duration before injury but longer ($P < 0.05$) than that during treatment with other medications. Performance returned to baseline levels within 10 calendar days of the initiation of dexamethasone therapy, and performance remained consistent with the preinjury baseline value after discontinuation of treatment and for months thereafter.

Figure 2 (right panel) shows the mean response force averaged across the last 7 sessions of the preinjury baseline, the first 7 sessions after the injury, the 7 sessions during dexamethasone treatment, and the 7 sessions after discontinuation of the dexamethasone treatment. One-way repeated-measures ANOVA revealed a significant effect of injury condition on force emitted during the TRD task ($F[3,18] = 21.623$, $P < 0.001$). Baseline, dexamethasone treatment, and posttreatment performances were equivalent with respect to mean response force. In contrast, mean response force during the injury period was significantly ($P < 0.05$) less than that during the baseline, dexamethasone treatment, and posttreatment periods. The pattern of behavioral recovery strongly suggests that the injury responded to dexamethasone therapy, an observation consistent with a nerve-related injury.

Discussion

The present case study demonstrates that an injury can be characterized and assessed objectively through performance of a well-trained operant task. Moreover, such performance served as a useful guide in evaluating treatment efficacy, putative pain relief, and complete recovery of function. By working together, veterinary and investigative personnel can improve detection, treatment evaluation, and general health monitoring by examining trained operant behavior. Behavioral testing is perhaps underutilized as a means of daily health monitoring because of the time, expense, and expertise required to conduct such tests. However, in animals already trained in such tasks, analysis of objective behavioral data can support assessment of animal welfare.

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